Clinical and genetic aspects of Marfan syndrome and familial thoracic aortic aneurysms and dissections

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An unanticipated copy number variant of chromosome 15 disrupting *SMAD3* reveals a three-generation family at serious risk for aortic dissection

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Abstract

Several genes involved in the familial appearance of thoracic aortic aneurysms and dissections (FTAAD) have been characterized recently, one of which is *SMAD3*. Mutations of *SMAD3* cause a new syndromic form of aortic aneurysms and dissections associated with skeletal abnormalities. We discovered a small interstitial deletion of chromosome 15, leading to disruption of *SMAD3*, in a boy with mild mental retardation, behavioral problems and revealed features of the aneurysms-osteoartritis syndrome (AOS). Several family members carried the same deletion and showed features including aortic aneurysms and a dissection. This finding demonstrates that haploinsufficiency of *SMAD3* leads to development of both thoracic aortic aneurysms and dissections, and the skeletal abnormalities that form part of the aneurysms-osteoarthritis syndrome. Interestingly, the identification of this familial deletion is an example of an unanticipated result of a genomic microarray and led to the discovery of important but unrelated serious aortic disease in the proband and family members.

Introduction

Several genes are known to cause syndromic and non-syndromic forms of aneurysms and dissections including *FBN1*, *TGFBR1*, *TGFBR2*, *ACTA2*, *MYH11* and *MYLK*. These genes encode either structural proteins associated with connective tissue, members of the TGF-β pathway or components or regulators of the contractile unit of vascular smooth muscle cells (SMCs). Recently, mutations in *SMAD3* were shown to cause a new syndromic form of aortic aneurysms and dissections associated with skeletal abnormalities (1). The authors proposed the name aneurysms-osteoarthritis syndrome (AOS). SMAD3 is another member of the TGF- β pathway and is essential for TGF- β signal transduction (2).
We describe the molecular and clinical analysis of a three-generation family, including eight members with an interstitial deletion of the long arm of chromosome 15 containing *SMAD3*. Although the deletion was initially identified in the proband, who showed mild mental retardation, it was not related to this phenotype. Unforeseen findings resulting from untargeted diagnostic testing such as microarray analysis or whole genome sequencing will be encountered with increasing frequency and will soon represent a major

challenge for counselors. In the case of the family described here, we were able to identify additional at-risk family members and could offer them appropriate cardiovascular follow-up and treatment options.

Patients and methods

Patients

The (simplified) pedigree of the family is depicted in Fig. 1. The proband (IV-1) was a boy with mild mental retardation and behavioral problems who was eligible for SNP-array analysis as part of mental retardation screening. Following the detection of two copy number variants (CNVs), family members were approached and asked to participate in SNP-array analysis. As one of the CNVs disrupted the gene *SMAD3*, which is associated with vascular and skeletal abnormalities, affected family members underwent a thorough physical and cardiologic examination, including transthoracic echocardiography (TTE) and magnetic resonance imaging (MRI), and a skeletal survey of the hands, elbows, spine, hips, knees and feet.

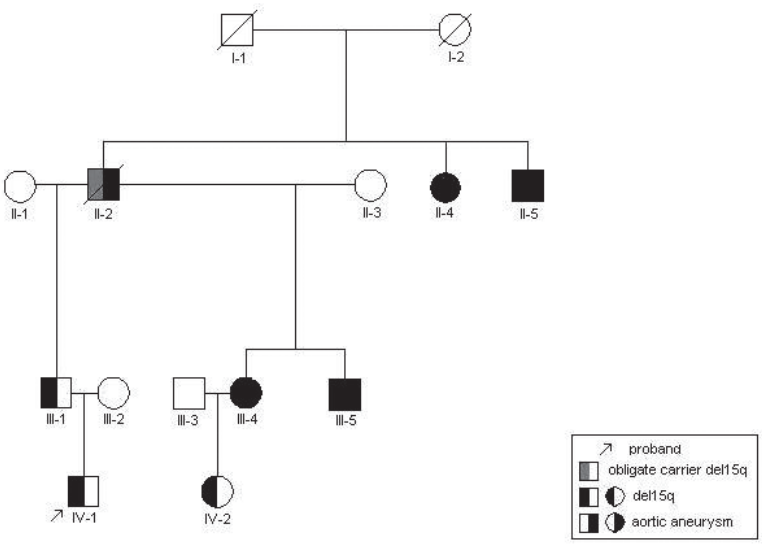

Figure 1. Pedigree of the family. Unaffected family members and family members who were not investigated are not shown.

High density microarray analyses, SNP arrays

The Affymetrix GeneChip Human Mapping 250K *NspI* array (Affymetrix, California, USA) contains 262.000 25-mer oligonucleotides with an average spacing of approximately 12 kb. Subject DNA (250 ng) was processed according to the manufacturer's instructions (www.Affymetrix.com). SNP copy number was assessed using the software program Copy Number Analyzer for Genechip (CNAG) Version 2.0 (3).

Transthoracic echocardiography

The diameters of the thoracic aorta at the level of the sinus of Valsalva, sinotubular junction and ascending aorta were measured in the left parasternal long axis view from leading edge-to-leading edge at end diastole, according to the recommendations of the American Society of Echocardiography (4). Body surface area (BSA) was calculated according to the DuBois formula (BSA (m^2) = 0.007184 × height (cm) 0.725 × weight (kg) 0.425). Measured values for adults were plotted against nomograms derived from individuals with normal cardiac findings and related to gender, age and body surface area (5). For children, Z-scores of the aortic root and ascending aorta were obtained from body surface area-related nomograms derived from normal children (6).

Magnetic resonance imaging

Imaging of the entire aorta and large arteries, including the cerebral arteries, was performed in the five adult family members (Fig. 1, II-4, II-5, III-1, III-6 and III-4).

Magnetic resonance (MR) imaging was performed on a 1.5 Tesla scanner (Philips Intera, Philips Medical Systems, Best, the Netherlands). Survey images were used for planning the scans. Body coil was used for MR angiography and 30 mL of a gadolinium-containing contrast agent (Dotarem; gadoteric acid 0.5 mmol/mL, Guerbet, Aulnay-sous-bois, France) was injected in an antecubital vein with an injection speed of 2 mL/sec. After bolus timing, MR angiography of the aorta was acquired during breath-hold. Scan parameters: 3D high resolution T1-fast field echo sequence, 75 mm coverage, field of view (FOV) 500 mm, repetition time (TR) 4.6 ms, echo time (TE) 1.3 ms, flip angle (FA) 40°, reconstructed voxel size 0.98 x 0.99 x 1.5 mm^3. Imaging of the carotid arteries (from aortic arch to head including circle of Willis) was performed using a head-neck coil and a 3D time-of-flight sequence. Scan parameters: FOV 250 mm, TR 21 ms, TE 6.9 ms, FA 20°. Reconstructed voxel size 0.82 x 0.82 x 1.0 mm^3.

Skeletal survey

Six family members underwent a radiographic skeletal survey of the whole spine, hands, elbows, knees (in weight-bearing position), ankles and feet. Osteoarthritis was

scored as positive when intervertebral disc space or joint space narrowing, osteophytes or reactive sclerosis was present. Presence of osteochondritis dissecans, spondylolysis, spondylolisthesis and scoliosis were also scored.

Results

SNP-array analysis of the proband (Fig. 1, IV-1) revealed two CNVs. The first variant was an interstitial duplication of the long arm of chromosome 22q11.2, of a maximum 3.2 Mb (130 SNP probes), from 17.020.301 bp to 20.258.915 bp (Ensembl release 54). The second variant was an interstitial deletion of the long arm of chromosome 15q22.3q23, of a maximum 194.8 kb (11 SNP probes), from 65.195.296 bp to 65.390.067 bp (Ensembl release 54) (Fig. 2a, b). The deleted region contained the three protein-encoding genes, *SMAD3*, *AC012568.7* and *IQCH* (Fig. 2c), and the proximal breakpoint was in intron 1 of *SMAD3*, resulting in the deletion of the gene from exon 2 onwards.

The proband's father (III-1) had the same copy number abnormalities as his son, and a further five family members (II-4, II-5 III-4, III-5 and IV-2) carried the interstitial deletion of chromosome 15q22.3q23 but not the interstitial duplication of chromosome 22q11.2 (Table 1).

The clinical features of the family are summarized in Table 1. Seven family members were investigated, including the proband and a 4-year-old child. Despite the fact that he could not be examined, the proband's grandfather (II-2) is an obligate carrier of the chromosome 15 deletion. The great-grandparents of the proband (I-1 and I-2) died at the age of 89 and 92 of unrelated disorders.

Of the five adults investigated, four experienced an aneurysm of the thoracic aorta. All four showed dilation of the aortic root, with three also showing dilation of the ascending aorta. MR imaging of patients II-4, II-5 and III-1 showed clear tortuosity (due to elongation) of the aorta, the carotid arteries and in III-1, of the superior mesenteric artery. The MR-angiography images of II-4 and II-5 (brother and sister) are depicted in Fig. 3a, b. No aneurysms or clear tortuosity of the cerebral arteries were detected. Although the 12-year-old proband and his 4-year-old cousin (IV-1 and IV-2) showed normal absolute aortic diameters, the Z-scores of the ascending aorta were higher compared to the Z-scores of the sinotubular junction. All five adult family members investigated for skeletal abnormalities showed scoliosis and four also exhibited intervertebral disc space narrowing. One family member (II-5) also had a grade one spondylolisthesis at lumbar level 2-3, secondary to facet artrosis. X-rays of the hands and spine (Fig. 3c-f) of a 68-year-old woman, II-4, showed both severe deforming osteoarthritis in the distal and proximal interphalangeal joints, without degeneration

of the scaphotrapezotrapezoidal (STT) and first carpometacarpal (CMC1), and severe degeneration of the whole spine. Neither spondylolysis nor ostechondritis dissecans was found in the investigated family members.

Apart from varicosis in III-1 and a soft skin in II-4, no skin abnormalities were found, and in particular, thread veins were absent. An abnormal uvula or a high narrow palate was found in four family members. Two family members showed inguinal hernias, including one that was recurrent (II-5) but possibly due to work-related physical exertion. Diaphragmatic hernias were detected by MRI in two patients (II-4 and II-5).

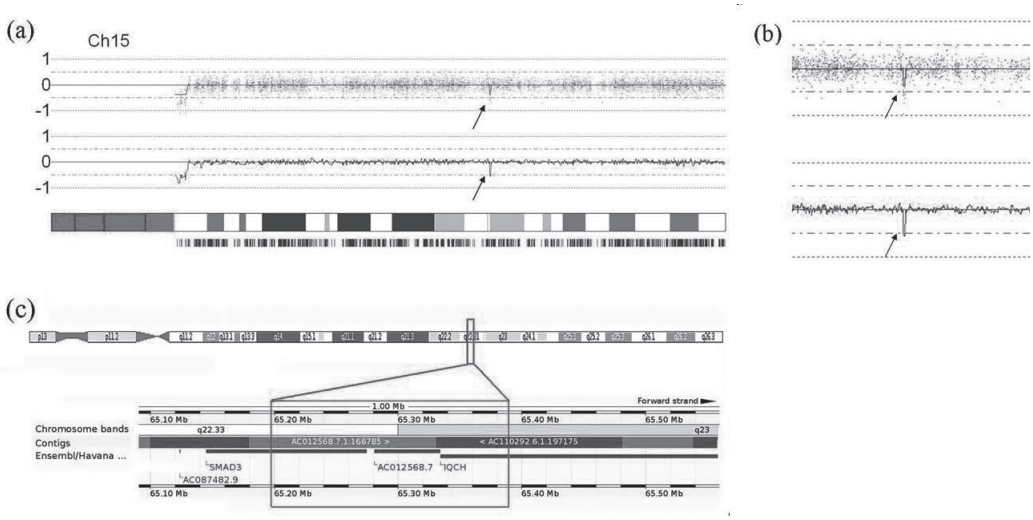

Figure 2. SNP array results – proband. (a) Chromosome 15 plot obtained by SNP array analysis, with black arrows indicating the deletion. (b) Detailed view of the 15q22.3q23 deletion. (c) Position of the deletion on chromosome 15 (Ensemble release 54, May 2009). The three known protein-encoding genes affected by the deletion, SMAD3, AC012568.7 and IQCH , are represented by the bars beneath the chromosome.

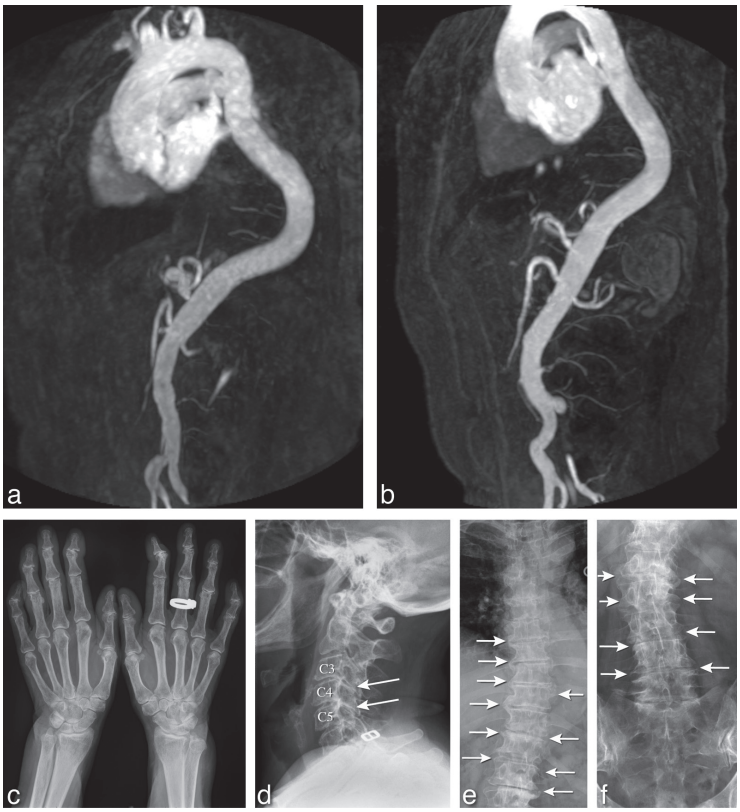

Figure 3. (a, b) Gadolinium contrast agent enhanced magnetic resonanceangiography (a, b) in a 69-year-old female patient (a, II-4)) and 64- year-old male patient (b, II-5); sister and brother. Both had severe elongation of the descending aorta with similar presentation of leftbackward bulging of the aorta (a, b). Both showed elongation of the carotid artery on the left side (not shown). (c–f) Conventional X-rays of the 69-year-old female patient (II-4) with osteoarthritic changes of the hands, showing osteoarthritic changes with narrowing of multiple interphalangeal joints and subluxation of distal interphalangeal joints 2–5 on left and 2, 3, 5 on right (c). No degenerative changes in first carpometacarpal or scaphotrapezotrapezoidal joints of wrists. Upper part of cervical spine showing narrowing of intervertebral disk spaces C3-4 and C4-5 and facetarthrosis (d, arrows). Thoracic (e) and lumbar (f) spine showing scoliosis convex to the left on thoracic level and to the right on lumbar level. Narrowing of intervertebral disk spaces and extensive degenerative spondylophytes on thoracic and lumbar levels (arrows).

Table 1. Laboratory investigations and clinical features

Pedigree number	II-2	III-1	IV-1 (proband)	III-4	IV-2	III-5	II-4	II-5
sex	M	M	M	F	F	M	F	M
age at examination; d: age at death	d54	42	12	33	4	18	69	64
BSA	u	2.2	1.9	1.9	0.8	2.3	1.9	1.9
Molecular results								
dup22q	ni	Yes	Yes	No	No	No	No	No
del15q (including *SMAD3*)	ni[1]	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Cardiovascular								
dilatation aortic root (cm)*	>2[2]	No (3.8)	No (2.9)	Yes (3.8)	No (2.1)	Yes (4.3)	Yes (4.0)	Yes (4.3)
dilatation ascending aorta (cm)*	Yes[2]	No (3.5)	No[3] (2.9)	No (2.9)	No[3] (2.0)	Yes (3.4)	Yes (4.7)	Yes (4.2)
dissection ascending aorta	Yes	No	No	No	No	No	No	No
aneurysms of other arteries	u	No	ni	No	ni	No	No	No
arterial tortuosity	u	Yes	ni	No	ni	No	Yes	Yes
mitral valve abNormalities	No	No	No	No	No	No	No	No
other heart disease	ihd	No	No	No	No	No	No	No
Skeletal								
pectus deformity	u	No	No	No	No	No	No	No
scoliosis	u	No[4]	Yes	Yes	No	Yes	Yes	Yes
joint laxity	u	No	No	No	No	Yes	No	No
Joints								
intervertebral disc degeneration	u	Yes	No	Yes	ni	No	Yes	Yes
osteoarthritis (location)	u	No	No	No	ni	No	Yes (hand, knee, hip)	Yes (hand, ankle, elbow, hip)
osteochondritis dissecans	u	No	No	No	ni	No	No	No
Craniofacial								
hypertelorism	u	Yes	Yes	No	No	No	No	No
abNormal palate or uvula	u	Broad uvula	Mild cleft uvula	High and narrow palate	No	No	No	Broad uvula
Skin/hernia's								
velvety skin	u	No	No	No	No	No	Yes	No
umbilical or inguinal hernia	u	No	No	No	No	No	Yes	Yes[5]
diaphragmatic hernia	u	No	No	No	ni	No	Yes	Yes
thread veins	u	No	No	No	No	No	No	No
varicosis	u	Yes	No	No	No	No	No	No
Other	u	Kyphosis; hearing deficit; atheroma cysts; learning difficulties; flexible hip joints; hyperkyphosis	Behavioral problems; slow motor and mental development; learning difficulties; ADHD	Striae	Strabismus	Learning difficulties; ADHD; striae		Sponylolisthesis L2-L3

*) measurements obtained by TTE; 1) obligate carrier; 2) observation during operation; 3) the Z-score of the ascending aorta was wider than the Z-score of the aortic root; 4) mild deviation of the spine; 5) recurrent inguinal hernias associated with physical labor. Abbreviations: u, unknown; ni, not investigated; ihd, ischemic heart disease; ADHD, attention deficit hyperactivity disorder.

Discussion

In this report we describe an incidental finding from an unrelated microarray analysis that led to the discovery of a serious health risk for the family involved. Microarray analysis is widely used in the diagnostic work-up of patients with mental retardation, with or without congenital abnormalities, and while much emphasis is placed in the literature on the likelihood of a pathogenic role for a CNV in the context of the patient's phenotype, the use of whole genome analysis in a diagnostic setting may detect variants unrelated to current clinical findings (7, 8). The identification of the family described here was due to a SNP-array analysis carried out as a component of mental retardation screening. The duplication of chromosome 22q11.2, found in both the proband and his father, is a frequently encountered CNV and has been associated with an extremely variable phenotype ranging from completely normal to mental retardation and congenital abnormalities. The duplication is often encountered in a parent with no or a mild phenotype (9, 10) and it is thus probable that the learning and behavioral problems of the proband are, at least in part, due to this duplication.

The interstitial deletion of chromosome 15q22.3q23 disrupts *SMAD3*, and excepting exon 1, leads to deletion of the whole coding region of *SMAD3* and haploinsufficiency. As recent reports demonstrated the role of mutations in *SMAD3* in an autosomal dominant syndromic form of aneurysms and dissections (1, 11, 12), it was decided to inform the family of the possible risk for aortic disease and to initiate a family study. In addition to the proband, seven other family members carried the deletion, including one obligate carrier. In addition to *SMAD3*, the deletion encompasses the genes *AC012568.7* and *IQCH* and has not been previously described in the normal population. All carriers showed vascular involvement and the majority also showed skeletal involvement. It is not clear whether the diaphragmatic hernia found in two carriers is due to the *SMAD3* deletion (Table 2).

Our findings are in accordance with those of previously described *SMAD3* families (1, 11, 12), including features of AOS with highly variable expression ranging from very mild features to dissection of the thoracic aorta. Thirteen *SMAD3* mutations have been described to date and are summarized in Table 2 (1, 11, 12, 13). Three reports have described 12 mutations in 11 families, with all cases recruited from TAAD families, implying a bias towards an aortic phenotype (1, 11, 12). This is further emphasized by the patient group described by van de Laar et al. (12), of which 89% showed cardiovascular anomalies, predominantly thoracic aortic aneurysms but also including high percentages of aneurysms of the abdominal aorta, large arteries and cerebral arteries. Though not ascertained due to aortic disease, the family described here shows aortic involvement in five of the six adults carrying the

Table 2. Summary of *SMAD3* mutations described in the literature

Nucleotide change	Amino acid change	Exon	Predicted effect	Family	Number of carriers	Number of obligate carriers	Phenotype	Reference
A>T	p.Asn197Ile	4	proline rich linker region; probable NMD	spor case	1	0	Knee OA no investigations for other features of AOS	Yao 2003
c.859C>T	p.Arg287Trp	6	MH2 domain	Family 1	25	5	TAAD, AAA, ICA, IAA, OA, aortic and arterial tortuosity	van der Laar 2011; van de Laar 2012
c.741–742delAT	p.Thr247ProfsX61	6	MH2 domain: premature stop in exon 7; proven NMD; removes nearly complete MH2 domain, TGFBR1 target site and residues involved in homo and heterodimer formation	Family 2	2	1		
c.782C>T	p.Thr261Ile	6	MH2 domain	Family 3	2	0		
c.313delG	p.Ala105ProfsX11	2	MH1 domain; probable NMD	Family 7	1	0		
c.539_540insC	p.Pro180ThrfsX7	4	proline rich linker region; probable NMD	Family 8	1	0		
c.788C>T	p.Pro263Leu	6	MH2 domain	Family 6	1	0		
c.1045G/C	p.Ala349Pro	8	MH2 domain	Family 4	1	0		
c.1080dupT	p.Glu361X	8	MH2 domain	Family 5	1	0		
c.652delA	p. Asn218fs	5	premature stop	TAA549	7	6	TAAD, AAA, ICA, IAA, OA, aortic tortuosity	Regaldo 2011
c.836G>A	p.Arg279Lys	6	MH2 domain, predicted to affect hydrogen bond formations and XPO4 interaction (promotion of SMAD3 nuclear transport)	TAA071	7	2		
c.836G>A	p.Arg279Lys	6	MH2 domain, predicted to affect hydrogen bond formations and XPO4 interaction (promotion of SMAD3 nuclear transport)	TAA072	2	0		
c.715G>A	p.Glu239Lys	6	MH2 domain, predicted to affect hydrogen bond formations	TAA365	3	1		
c.235C>T	p.Ala112Val	2	not conserved in fruitfly, possibly damaging	TAA115	4	1		

Abbreviations: AAA, abdominal aortic aneurysms; AOS, aneurysms-osteoarthritis syndrome; IAA, iliac arterial aneurysms; ICA, intracranial aneurysms; OA, osteoarthritis; TAAD, thoracic aortic aneurysms and dissections

SMAD3 deletion. In the 12-year-old proband (Fig. 1, IV-1) and a 4-year-old cousin (Fig. 1, IV-2), the abnormal feature of an ascending aorta wider than the sinotubular junction was seen and could be the first sign of future dilatation. All five adult carriers investigated showed remarkable scoliosis, including four with radiologically proven intervertebral disc degeneration. The 68-year-old woman (II-4) with severe distal osteoarthritis of the hands showed normal STT and CMC1 joints, in contrast to cases described in the literature (1). This might well be the classic form of osteoarthritis and not linked to the disruption of *SMAD3*.

As little is yet known of the two other genes in the deleted region, a possible role in the phenotype cannot be entirely ruled out. *AC012568.7* encodes a protein of unknown function and the *IQCH* gene is thought to be associated with tall stature (14).

We conclude that the similarities of the vascular and skeletal phenotype in the family described here to those of other families with mutations in *SMAD3* is the result of the disruption and resulting haploinsufficiency of *SMAD3*. The discovery of a serious health risk unrelated to the phenotype of the proband is a telling example of the potential impact of whole genome analysis on family members. Although current literature contains few articles describing unexpected results, this family underlines the importance of precise genetic interpretation of a CNV and the genes involved in order to provide appropriate information to the patient and family. The identification of at-risk members of this particular family allowed early detection of vascular disease, appropriate counseling and facilitated possibly life-saving follow-up and treatment.

Conflict of interest statement

None of the authors has a conflict of interest to declare.

Acknowledgments

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